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2018-07

Quarto , T , Paparella , I , De Tullio , D , Viscanti , C , Fazio , L , Taurisano , P , Romano , R , Rampino , A , Masellis , R , Popolizio , T , Selvaggi , P , Pergola , G , Bertolino , A & Blasi , G 2018 , ' Familial Risk and a Genome-Wide Supported DRD2 Variant for Schizophrenia Predict Lateral Prefrontal-Amygdala Effective Connectivity During Emotion Processing ' , Schizophrenia bulletin , vol. 44 , no. 4 , pp. 834-843 . <https://doi.org/10.1093/schbul/sbx128>

<http://hdl.handle.net/10138/304064>

<https://doi.org/10.1093/schbul/sbx128>

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Familial Risk and a Genome-Wide Supported *DRD2* Variant for Schizophrenia Predict Lateral Prefrontal-Amygdala Effective Connectivity During Emotion Processing

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The brain functional mechanisms translating genetic risk into emotional symptoms in schizophrenia (SCZ) may include abnormal functional integration between areas key for emotion processing, such as the amygdala and the lateral prefrontal cortex (LPFC). Indeed, investigation of these mechanisms is also complicated by emotion processing comprising different subcomponents and by disease-associated state variables. Here, our aim was to investigate the relationship between risk for SCZ and effective connectivity between the amygdala and the LPFC during different subcomponents of emotion processing. Thus, we first characterized with dynamic causal modeling (DCM) physiological patterns of LPFC–amygdala effective connectivity in healthy controls (HC) during implicit and explicit emotion processing. Then, we compared DCM patterns in a subsample of HC, in patients with SCZ and in healthy siblings of patients (SIB), matched for demographics. Finally, we investigated in HC association of LPFC–amygdala effective connectivity with a genome-wide supported variant increasing genetic risk for SCZ and possibly relevant to emotion processing (*DRD2* rs2514218). In HC, we found that a “bottom-up” amygdala-to-LPFC pattern during implicit processing and a “top-down” LPFC-to-amygdala pattern during explicit processing were the most likely directional models of effective connectivity. Differently, implicit emotion processing in SIB, SCZ, and HC homozygous for the SCZ risk rs2514218 C allele was associated with decreased probability for the “bottom-up” as well as with increased probability for the “top-down” model. These findings suggest that task-specific anomaly in the directional flow of information or disconnection between the amygdala and the LPFC is a good candidate endophenotype of SCZ.

Key words: endophenotype/*DRD2* rs2514218/dynamic causal model/implicit emotion processing/explicit emotion processing

Introduction

Notwithstanding the well-established link between emotional anomalies and SCZ,¹ the brain functional mechanisms translating genetic risk for this brain disorder into these clinical symptoms have not been fully characterized yet.^{2–4} For example, a number of studies investigating regional brain activity in patients revealed reduced recruitment of the amygdala in response to emotional stimuli,^{5–7} while others indicated intact or even greater recruitment of this brain region.^{8–10} Furthermore, reduced^{11,12} or greater¹³ prefrontal cortex activation in patients with SCZ compared with healthy controls (HC) have been reported during different emotional tasks.

A factor possibly contributing to such inconsistencies is that the physiology of emotion processing includes different components, which may elicit different and complex brain patterns.^{14,15} For example, a more automatic and intuitive (implicit) processing of emotional stimuli appears to be mainly mediated by the amygdala, while explicit emotional evaluation prominently engages the prefrontal cortex, including its lateral portion.^{16–19} Importantly, these brain regions are functionally connected, as indicated previously.^{20–23} In this regard, animal and human studies suggest that different patterns of brain functional connectivity as a function of specific subcomponents of emotional processing are likely.^{24,25} Previous work proposes that limbic regions modulate cortical

areas during automatic processing of emotional stimuli, whereas the direction of the modulation is inverted during explicit emotional evaluation or regulation.²⁶ Overall, this earlier body of work suggests that it is crucial to investigate the reciprocal functional influence of limbic and cortical regions and how it relates to different components of emotion processing. This investigation is key to identify putative anomalies in the functional relationship between brain regions in SCZ. Consistent with this perspective, well credited models have suggested that anomalies in the influence of dopamine receptors on NMDAR-mediated changes in synaptic efficacy^{27,28} may subtend altered brain functional integration, or “disconnection,” in SCZ.^{28–34} Accordingly, previous studies in SCZ reported altered functional connectivity between the amygdala and prefrontal regions during emotion processing.^{4,35–39}

Another factor possibly leading to the lack of identification of reproducible and key brain correlates of the processing of emotions in SCZ is that brain activity in patients may be confounded by state variables, including pharmacological treatment and levels of symptoms.^{40–42} A strategy to overcome this issue is the study of healthy siblings of patients (SIB) with schizophrenia, who share on average 50% of genetic variation with probands and their brain activity is not affected by state variables. Thus, investigation of these individuals is a first step in disambiguating if and how anomalous processing of emotions is linked with risk for SCZ. However, only few studies have been performed to date using this approach and they have reported inconsistent results.^{40,43–45} In particular, previous findings in SIB suggest that the functional coupling between the amygdala and the cingulate cortex during implicit processing of emotional faces is not a trait phenotype of SCZ.⁴⁰ On the other hand, other results obtained with effective connectivity approaches in unaffected first-degree SCZ relatives provide opposite evidence. For example, a study in adolescent offspring of SCZ indicated reduced effective connectivity between the amygdala and the prefrontal cortex as measured with dynamic causal modeling (DCM).⁴⁶ Similarly, a more recent study³⁸ revealed reduced graph-based connectivity during emotional face processing in a subnetwork including the limbic and visual cortex as well as the pallidum and the thalamus in relatives of SCZ compared with controls. Thus, different functional connectivity approaches may lead to inconsistent findings across studies. Overall, the paucity and the inconsistency of the results in this field call for further investigation.

Another important point is that the investigation of anomalies in emotion processing in SIB is only a first step in order to identify emotion-related brain endophenotypes for the disorder. To further support the utility of such phenotypes for genetic investigations, another step is the study of their relationship with genetic variants increasing risk for SCZ. In this regard, it is well known that dopamine and the dopaminergic D2 receptor are

crucial for emotion processing,^{47–49} modulate physiology of the amygdala and the prefrontal cortex,^{50–52} and have been strongly implicated in SCZ.⁵³ Furthermore, variation within the D2 gene (*DRD2*) has been associated with emotional phenotypes.⁵⁴ Moreover, the largest genome-wide association study to date indicated that the C allele of a single nucleotide polymorphism (SNP) in close proximity to the D2 coding gene *DRD2* (rs2514218 C/T) is associated with diagnosis of SCZ,⁵⁵ and with SCZ-related phenotypes.^{56,57}

The aim of this study is to investigate the association of familial risk and of a genome-wide supported variant increasing risk for SCZ with patterns of brain functional effective connectivity during emotional processing. Given that inconsistencies of previous reports in SCZ^{7,10,38,40,46} may be explained in part by the different components involved in emotion processing, we separately investigated implicit and explicit emotional processes. Moreover, unlike other recent studies,³⁸ we focused on effective connectivity between the amygdala and the lateral prefrontal cortex (LPFC), which are brain regions functionally coupled,^{20–23} modulated by dopamine,^{50–52} previously associated with SCZ,^{5–7,9–13} and strongly involved in emotion processing.^{19,58,59} With this aim, we used DCM, which is an effective connectivity approach describing how the present state of one neuronal population causes dynamics in another neuronal population and how this interaction changes under the influence of external perturbations (eg, experimental manipulations) or brain activity.⁶⁰ Thus, this approach is not affected by the limitations of other methodologies addressing brain functional connectivity which do not account for directionality, influence, or causality between interacting regions.⁶¹ Therefore, it is well suited for unveiling directionality of cortico-limbic connectivity during implicit and explicit emotional processes.

Here, we first investigated patterns of LPFC–amygdala effective connectivity during emotion processing in a sample of HC to establish the physiology of these networks. Then, we studied the putative modifying effect of familial risk for SCZ on effective connectivity patterns. Finally, to further support the utility of these phenotypes for genetic studies, we investigated whether *DRD2* rs2514218 affects dynamics of effective connectivity. We hypothesized that effective connectivity between the amygdala and the LPFC during emotion processing may be modulated by risk for SCZ. In particular, we hypothesized that SCZ and SIB may exhibit a similar alteration of physiological models of amygdala–LPFC effective connectivity and that *DRD2* variation might be associated with such anomaly.

Materials and Methods

Participants

Two hundred seventeen HC were included in the study in order to address physiological patterns of effective

connectivity during implicit and explicit processing of facial emotions (table 1). Furthermore, 56 of these HC, 36 SIB, and 40 SCZ were included to address the relationship between LPFC–amygdala effective connectivity and familial risk for SCZ in groups with similar N and matched demographics. In greater detail, subsamples of HC were iteratively and randomly generated using Excel from the larger group. Furthermore, iterative *t*-tests were performed on such subsamples using Excel in order to compare their demographics (ie, handedness, gender, age, socioeconomic status, and intelligence quotient) with those of SCZ and SIB. Such iterations were stopped when they provided a subsample of HC in which demographics were matched with those of the other diagnostic groups (all $P > .05$) (table 1). DNA samples were also available for 151 individuals of the total sample of HC. These subjects were genotyped for *DRD2* rs2514218. After genotyping, there were

39 subjects with the TT genotype for this SNP, whose handedness, gender, age, and socioeconomic status were used to randomly identify with the procedures described above matched 39 CT and 39 CC individuals (all $P > .05$) (table 1).

All participants were white Caucasians from the region of Puglia, Italy. The Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders IV was used to confirm diagnosis of SCZ for patients and to exclude any psychiatric disorder for SIB and HC. All SCZ had been on stable pharmacological treatment with first or second generation antipsychotics for at least 8 weeks before entering the study. Exclusion criteria and other details are specified in the supplementary material. All subjects provided written informed consent to the study after the procedure had been fully explained to them. The present study was approved by the local Institutional Review Board.

Table 1. Characteristics (Mean \pm Standard Error) of the Samples Used in This Study

Characterization of LPFC–amygdala effective connectivity in HC					
HC (<i>n</i> = 217)					
Age, years	26.1 (6.6)				
Gender, <i>n</i>					
Male	105				
Female	112				
Handedness	0.7 (0.5)				
Hollingshead index	37 (17.3)				
IQ	107.7 (16.1)				
Effect of familial risk for schizophrenia on LPFC–amygdala effective connectivity					
	HC (<i>n</i> = 56)	SIB (<i>n</i> = 36)	SCZ (<i>n</i> = 40)	Yates-corrected χ^2	<i>P</i>
Age, years	31.4 (10.4)	35.4 (10.1)	33.2 (8.5)		NS
Gender, <i>n</i>					
Male	30	13	24	3.42	NS
Female	26	23	16		
Handedness	0.8 (0.4)	0.8 (0.5)	0.8 (0.5)		NS
Hollingshead index	29.6 (12.9)	28.6 (15.3)	28.2 (14.4)		NS
Premorbid IQ	111 (5.3)	108.8 (8.9)	108.1 (7.8)		NS
Chlorpromazine equivalents			536 (249)		
PANSS total score			72.7 (15.5)		
Association of <i>DRD2</i> rs2514218 with LPFC–amygdala effective connectivity					
	CC (<i>n</i> = 39)	TC (<i>n</i> = 39)	TT (<i>n</i> = 39)	Yates-corrected χ^2	<i>P</i>
Age, years	27.23 (5.87)	27.18 (8.27)	27.44 (7.84)		NS
Gender, <i>n</i>					
Male	20	17	18	<0.001	NS
Female	19	22	21		
Handedness	0.87 (0.41)	0.77 (0.58)	0.87 (0.41)		NS
Hollingshead index	39.88 (15.28)	42.63 (16.15)	40.59 (17.61)		NS
IQ	108.51 (11.21)	109.95 (10.75)	108.2 (8.24)		NS

Note: IQ, Intelligence quotient; HC, healthy controls; SCZ, schizophrenia patients; SIB, unaffected siblings of schizophrenia patients; NS, not significant; PANSS, Positive and Negative Syndrome Scale.

Genotyping

Subjects were genotyped for *DRD2* rs2514218 (see supplementary material). Based on this polymorphism, 39 subjects were TT, 60 CT, and 52 CC. The CC and CT groups were downsized to match the TT group as above reported.

Functional Magnetic Resonance Imaging Task

The event-related functional magnetic resonance imaging (fMRI) task^{15,45,62–64} consisted of 2 runs, each presenting angry, fearful, happy and neutral facial expressions from a validated set of facial pictures (NimStim, <http://www.macbrain.org/resources.htm>).⁶⁵ During one run (emotional perceptual processing—implicit processing), subjects identified the gender of each face. In the other run (explicit emotional evaluation—explicit processing), they had to decide if they would like to “approach” or “avoid” the face (supplementary material).

Demographic, Clinical, and Behavioral Data Analysis

ANOVAs and χ^2 were used to assess demographic differences between groups and to investigate the effect of diagnosis and genotype on behavioral data. *t*-Tests for independent samples were used for post hoc analyses.

Functional Magnetic Resonance Imaging Data Acquisition and Analysis

Blood oxygen level dependent (BOLD) fMRI was acquired on a GE Signa 3T scanner while participants performed the task (supplementary material). Analysis of the fMRI data was completed using Statistical Parametric Mapping 8 (SPM8, Wellcome Department of Cognitive Neurology) (supplementary material). Voxels associated with a main effect of emotion (all emotions vs fixation crosshair) in the large sample of HC (family wise error voxel-wise corrected $P < .05$) (see supplementary table 1 for detailed statistics) were used for time series extraction in the DCM analysis (see below).

Effective Connectivity

We used DCM (version 10) as implemented in SPM8 to investigate LPFC–amygdala effective connectivity.⁶⁶ In DCM, regional time series derived from a GLM analysis are used to analyze connectivity and its modulation by experimental conditions. DCM models hidden neuronal dynamics and the influence that one neuronal system exerts over another.⁶⁶ It allows modeling of the endogenous coupling between 2 regions, which is context independent (“intrinsic connections”). The impact of experimental stimuli can be modeled directly on specific regions (“driving input”) or on the strength of coupling between 2 regions (“modulatory input”). In DCM, the modeled neuronal dynamics are transformed into a measured response—the BOLD signal—using a hemodynamic forward model.⁶⁶

Time Series Extraction. Three brain regions crucially involved in processing of emotional faces^{20–23} were included in the model: the primary visual cortex (V1) (as the region of access of visual stimuli), the amygdala, and the LPFC. Regional time series were extracted at the single-subject level using a combination of functional and anatomical criteria. In particular, the voxel-wise FWE corrected results of the GLM, investigating the main effect of emotion in the large sample of HC, were masked with anatomical regions of interest (ROIs) (Brodmann Area 17, amygdala, lateral superior, middle and inferior frontal gyrus) as defined with the Wake Forest University PickAtlas (<http://fmri.wfubmc.edu/cms/software#PickAtlas>). Then, the peak of activity resulting from this procedure was used as the center of an 8-mm radius sphere from which the first eigenvariate was extracted. Given that all peaks were located in the right hemisphere, we focused our analysis in this hemisphere only.

Model Space and Selection. The BMS analysis focused on the modulation of LPFC–amygdala effective connectivity by contextual stimuli (eg, facial expressions presented during implicit vs explicit emotional faces task). Two models were built assuming bilateral intrinsic connections between V1, the amygdala and the LPFC, as well as V1 as the driving input region. These 2 models differed from each other for the influence of the modulatory effects of facial expressions during implicit or explicit processing on the connections between the amygdala and the LPFC. In our first model (“bottom-up”), the modulatory input (all faces vs crosshair) impacted the connection from the amygdala to the LPFC. In the second model (“top-down”), the modulatory input impacted the connection from the LPFC to the amygdala (figure 1).

After model set up, random-effects Bayesian model selection (BMS) analyses were performed in order to calculate exceedance probabilities (EP) (ie, the probability that one model is more likely than another model) in each comparison of interest (ie, implicit vs explicit processing of facial expressions in (1) the total sample of HC; (2) the matched samples of HC, SCZ, SIB; and (3) *DRD2* rs2514218 TT, CT and CC of HC). All the BMS analyses were performed for the explicit and implicit run separately. In addition, we performed Bayesian model averaging (BMA) on the winning model, an alternative approach that allows for statistical comparison of parameters between groups (see supplementary material for details of this analysis and results).

Results

Characterization of LPFC–Amygdala Effective Connectivity in HC

BMS on the whole sample of 217 HC indicated that the “bottom-up” was the winning model during implicit

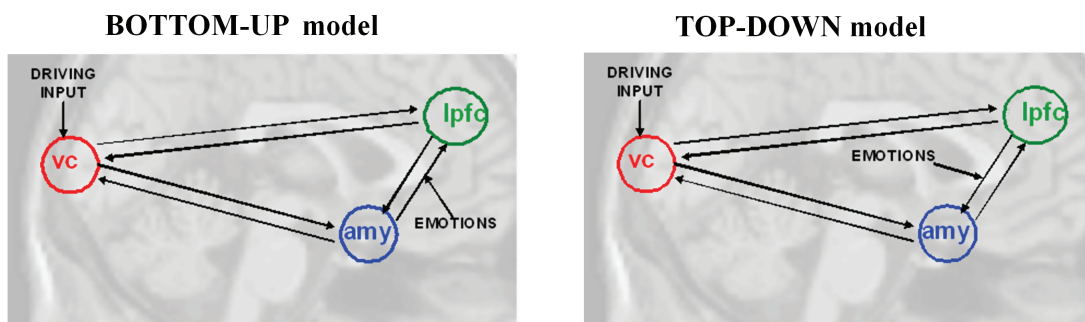


Fig. 1. Models of effective connectivity tested in the study.

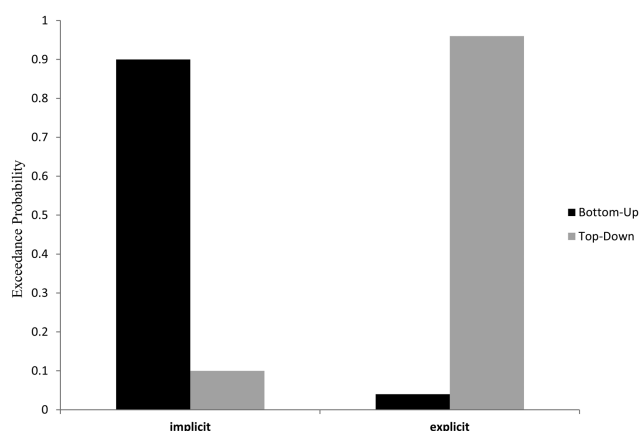


Fig. 2. Graph showing exceedance probability (EP) of the “bottom-up” and “top-down” models of effective connectivity between the amygdala and the LPFC in HC during implicit emotion processing and explicit emotional evaluation.

emotion processing. In this model, the modulatory effects were set from the amygdala to the LPFC. In particular, EP (0.90) of this model in HC was “positive” according to a widely used classification,⁶⁷ while EP of the top-down model was “not positive” (0.10).⁶⁷ Furthermore, BMS on the explicit run indicated that the “top-down” was the winning model in the same sample of HC. Here, the modulatory effects were set from the LPFC to the amygdala. EP (0.96) of this model was “strong”,⁶⁷ while EP of the bottom-up model was not positive (0.04) (figure 2).

Effect of Familial Risk for SCZ on LPFC–Amygdala Effective Connectivity

Further BMS was performed in the matched samples of HC, SIB, and SCZ. Results of BMS during implicit and explicit processing in HC were consistent with those performed in the larger sample. In particular, winning models were the “bottom-up” during implicit processing, (“bottom-up” EP = 0.73; “top-down” EP = 0.27), and the “top-down” during explicit processing (“bottom-up” EP = 0.33; “top-down” EP = 0.67) (figure 3A).

Differently, BMS results in SCZ revealed that the “top-down” was the winning model during implicit processing (“bottom-up” EP = 0.24; “top-down” EP = 0.76). Furthermore, the “top-down” was the winning model also during explicit processing (“bottom-up” EP = 0.31; “top-down” EP = 0.69) (figure 3A). As in SCZ, findings in SIB indicated that the “top-down” was the winning model during implicit (“bottom-up” EP = 0.39; “top-down” EP = 0.61) and explicit (“bottom-up” EP = 0.39; “top-down” EP = 0.61) processing (figure 3A). Overall, in both SCZ and SIB during implicit processing there was a lower probability for the “bottom-up” compared with the “top-down” model.

Behavioral Data

Behavioral data indicated a main effect of diagnosis on reaction time during implicit processing of facial expressions (mean \pm standard deviation: HC = 532.3 ± 122.5 ; SIB = 664.7 ± 127 ; SCZ = 645.2 ± 115.2) ($F_{2,124} = 16.06$, $P < .001$). Post hoc analysis indicated greater reaction time in SCZ and SIB compared with HC ($P < .001$), whereas no significant difference was present between SCZ and SIB ($P > .05$). Moreover, there was a diagnosis by emotion interaction ($F_{6,378} = 6.12$; $P < .001$) on the number of avoided faces during the explicit task. Post hoc analysis revealed that SCZ avoided more happy faces and less fearful and angry faces compared with both SIB and HC ($P < .04$). Further details of behavioral data are included in the supplementary material.

Association of DRD2 rs2514218 With LPFC–Amygdala Effective Connectivity

BMS results in TT and CT HC revealed that the “bottom-up” was the winning model during the implicit processing run (TT = “bottom-up”: 0.83; “top-down”: 0.17; CT = “bottom-up”: 0.98; “top-down”: 0.02), while the “top-down” was the winning model during the explicit processing run (TT = “bottom-up”: 0.07; “top-down”: 0.93; CT = “top-down”: 0.62; “bottom-up”: 0.38) (figure 3B). BMS results in CC HC (who are homozygous

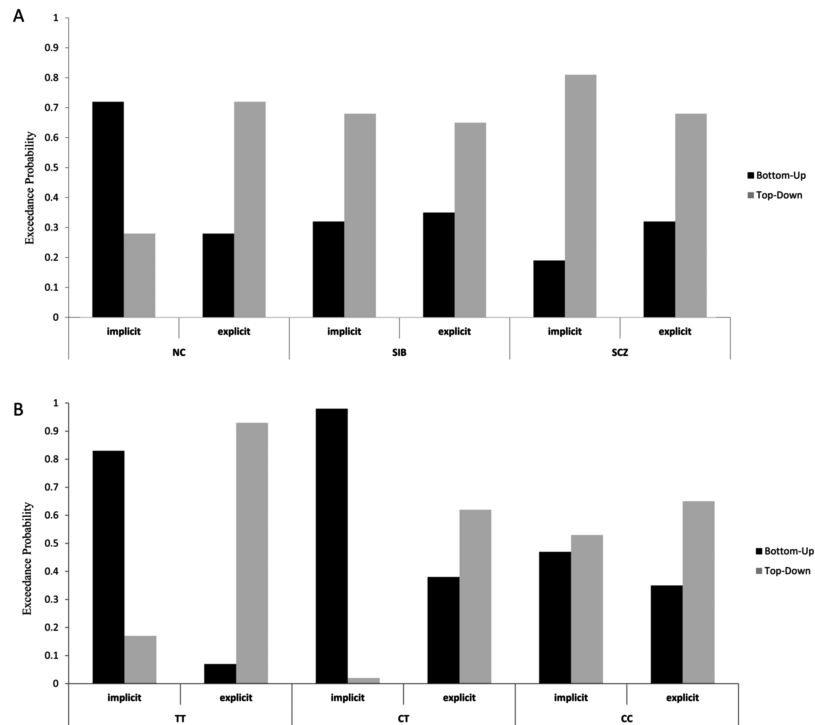


Fig. 3. Graphs showing (A) exceedance probability (EP) of the “bottom-up” and “top-down” models of effective connectivity between the amygdala and the LPFC in matched samples of HC, SCZ, and SIB. (B) Exceedance probability (EP) of the “bottom-up” and “top-down” models of effective connectivity between the amygdala and the LPFC as a function of *DRD2* rs2514218 in HC.

for the SCZ risk allele) indicated that the “top-down” was the winning model during explicit processing (“bottom-up”: 0.35; “top-down”: 0.65) (figure 3B). On the other hand, during implicit processing there was no clear winning model, but a slightly lower probability for the “bottom-up” compared with the “top-down” model in individuals homozygous for the risk C allele (“bottom-up”: 0.47; “top-down”: 0.53) (figure 3B). Finally, there was no effect of genotype on behavior during the task (all $P > .05$).

Given these effects of rs2514214, we also explored the LPFC–amygdala effective connectivity in the whole sample of HC, and in a sample of HC matched with SIB and SCZ, excluding individuals homozygous for the C allele. This investigation did not relevantly change the results (supplementary material).

Discussion

Our results indicate abnormal patterns of effective connectivity between the amygdala and the LPFC during different subcomponents of emotion processing in SCZ and in SIB. Furthermore, HC homozygous for the C allele of *DRD2* rs2514218, a genome-wide supported variant increasing genetic risk for SCZ,⁵⁵ display similar functional brain abnormalities. Overall, these findings suggest that familial risk for SCZ is associated with anomalous effective connectivity between the LPFC and

the amygdala especially during implicit processing of emotional stimuli. Furthermore, they suggest that a risk allele for SCZ confers liability for this brain functional abnormality.

The results that we found in SCZ, SIB, and HC homozygous for the C allele of *DRD2* rs2514218 should be read in light of our findings in the large sample of HC that we investigated. Here, the emotional task at hand differentially modulated the physiological effective connectivity between the amygdala and the LPFC during emotion processing. More in detail, there was a greater probability for an amygdala-to-LPFC effective connectivity during implicit, perceptual processing of emotional stimuli. On the other hand, explicit emotional evaluation was sustained by greater probability for the opposite pattern of effective connectivity between these brain regions. A possible interpretation of these findings may be based on the known primary role of the amygdala in perceptual processing of emotional stimuli⁵⁷ and of the LPFC in the explicit evaluation of emotional stimuli and emotional regulation.^{19,59} Also, several findings suggest that emotional stimuli imply a faster amygdala response compared to those of associative cortices, such as the LPFC.⁶⁸ Thus, it is possible that the amygdala acts as a first functional node in the processing of emotional information conveyed by our task involving implicit, perceptual processing. Then, the amygdala may send relevant inputs to the LPFC that exerts a role in integrating emotional

information in a more general context of information processing, as previously suggested.⁶⁹ Differently, the explicit emotional part of our task needs integration of emotional regulation, social and cognitive functions, which are more supported by the LPFC according to previous literature.^{17,19,59,69} Thus, a task-mediated flow of information from the LPFC to the amygdala may contribute to modulate more automatic responses sustained by the latter brain region when regulatory processes based on social and/or cognitive evaluation of emotional stimuli are required.

Our results indicate that effective connectivity is dysfunctional in both SCZ and in SIB. In greater detail, in both these groups of individuals the “top-down” model is more likely than the “bottom-up” model during both implicit and explicit emotion processing. Given that HC had the “bottom-up” as the winning model during implicit processing and the “top-down” as the winning model during explicit processing, these results indicate that the probability for a physiological model of effective connectivity in SCZ and SIB during implicit processing of emotional stimuli is not preserved. These findings suggest a task-specific breakdown of the physiological flow of information between the amygdala and the LPFC in SCZ. Importantly, they also suggest that the abnormal pattern of effective connectivity during implicit emotion processing is a crucial phenotype of SCZ and that it is associated with familial risk for the disorder, rather than with specific state variables, including pharmacological treatment and levels of symptoms. In this regard, these results are in line with models proposing altered brain functional integration as a key pathophysiological mechanism for SCZ,^{28,30–34} and consistent with studies reporting anomalous functional coupling between the amygdala and prefrontal regions in SCZ and in subjects at greater risk for this disorder.^{4,35–38,46,70,71} Furthermore, they converge with recent evidence indicating a decrease of the amygdala-to-LPFC effective connectivity during implicit emotion processing in SCZ compared to controls,³⁹ and are consistent with the view that this anomaly may subtend an inability to properly evaluate and/or regulate emotional inputs at the frontal level.³⁹ Interestingly, a well-credited model of SCZ proposes that symptoms of this brain disorder may be explained by the abnormal attribution of emotional salience to irrelevant stimuli,^{70,71} which is also consistent with our findings.

Intriguingly, we also found in HC a similar between-group modulation of LPFC–amygdala effective connectivity by *DRD2* rs2514218, which has been associated with diagnosis of SCZ in the largest genome wide association study to date.⁵⁵ Here, the alteration of patterns of effective connectivity in HC homozygous for the SCZ risk C allele was similar to those present in SIB and in SCZ. As in SCZ and SIB, we found that the physiological pattern of amygdala/LPFC effective connectivity in CC HC is altered during implicit emotion processing, such

that there was no winning model in this genotype group. On the other hand, the T allele predicted the physiological model of effective connectivity between these brain regions. Thus, these results are consistent with the likely involvement of D2 signaling in SCZ and with its relevance for emotion processing.⁵⁴ Moreover, they are also consistent with the above-mentioned model postulating abnormal attribution of emotional salience to irrelevant stimuli in SCZ,⁷² for which a dopaminergic dysregulation is key.⁷² More in general, they support the use of effective connectivity during emotion processing for genetic investigations aimed at identifying true endophenotypes for the disease.

Importantly, the effect of rs2514218 on molecular phenotypes related to D2 receptor is still not clear. For instance, a recent study⁵⁷ did not find any detectable relationship between this polymorphism and *DRD2* expression levels, suggesting that the molecular mechanisms of the present findings should be further investigated. On the other hand, recent work has found association of rs2514218 with striatal function in SIB,⁵⁷ as well as with response to antipsychotic treatment in patients,⁵⁶ supporting the relevance of this SNP for correlates of SCZ. However, in these previous studies heterozygous and homozygous individuals were collapsed in one group of C or T carriers to investigate genotype effects. This strategy does not allow to verify consistency of these previous findings with ours when considering the model of genetic association of rs2514218 with SCZ-related phenotypes.

Some potential limitations should be considered in this study. First, the moderately high level of premorbid functioning of the SCZ patients could limit the generalizability of the findings to other SCZ patients. Second, we used a relatively slow repetition time during fMRI acquisition compared with those attainable with multi-band methods. This limitation prevented us to obtain a lower temporal resolution. Third, DCM analysis did not allow us to consider in the statistical design physiological covariates. This limitation prevented us to completely over-ride these possible sources of noise. However, our investigation of temporal signal-to-noise ratio (see supplementary material) suggests that these physiological parameters do not strongly affect our results. Fourth, the design of the experiment did not allow us to directly compare implicit vs explicit models of effective connectivity. However, this comparison was not the major aim of our study, while our purpose was to investigate modulation of effective connectivity during different emotional processes as a function of risk for SCZ and of rs2514218 genotype. Fifth, the fixation crosshair used as a baseline in this study does not allow us to control for the activity or connectivity related to visual complexity, cognitive demand, and motor response of the emotional stimuli. However, it controls for activity or connectivity related to basic body functions (eg, breath, basic vision, space perception, body temperature regulation, etc.) while

preserving detection of brain connectivity during emotion processing. This approach may overcome some of the limitations associated with other baseline strategies and is consistent with the method used in several previous studies.¹⁴ Sixth, our model specification is a simplification of the complex brain network sustaining emotion processing and we may not exclude the putative relevance of other brain nodes or other possible model specifications in the context of our work. However, we focused our analyses on brain regions involved in emotion processing, consistently associated with SCZ and modulated by dopamine signaling. Finally, we focused on association of effective connectivity during emotion processing with a single genetic variation when SCZ is very likely associated with polygenic risk. This strategy does not allow a full understanding of the relationship between effective connectivity during emotion processing and the genetics of SCZ. On the other hand, we investigated association of rs2514218 with LPFC–amygdala effective connectivity to further support the utility of this phenotype for genetic studies relevant to SCZ.

In conclusion, our results suggest that altered LPFC–amygdala effective connectivity during emotion processing is a crucial correlate of SCZ, which may be further investigated as a promising candidate endophenotype for this brain disorder. In this regard, further studies should address the relationship between effective connectivity during emotion processing and polygenic scores indexing genetic risk for SCZ. Such investigation might shed more light on the pathophysiological underpinnings of emotional dysfunction of this brain disorder.

Supplementary Material

Supplementary data are available at *Schizophrenia Bulletin* online.

Funding

The present study has been financially supported by the European Community's Seventh Framework Programme (project number 602450; URL: http://ec.europa.eu/research/health/medical-research/brain-research/projects/imagemend_en.html). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

We wish to thank Dr Mariana Nair Castro for useful comments and great assistance during the revision process and Dr Linda Antonucci for many helpful discussions on the field of connectivity. We also would like to express our thanks to all the volunteers for having participated to the study.

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